ORIGINAL ARTICLE

α-SMA AS A MESENCHYMAL MARKER IN CHRONIC HEPATITIS C INFECTION .

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ABSTRACT

Background: Liver biopsy so far, is the mainstay of diagnosing hepatic fibrosis. Activated HSCs express certain mesenchymal markers, of which Smooth Muscle Actin is a well-known marker. This study aims to appreciate expression of Alpha Smooth Muscle Actin in hepatic stellate cells of CHC cases and to find out the frequency of α-SMA positive HSCs in perisinusoidal, periportal and pericentral regions of liver.

Methods: Liver biopsies of sixty Chronic Hepatitis C cases were immmunostained using anti α-SMA antibody. To assess immunoexpression, semi quantitative scoring was done in three areas in each sample; pericentral, periportal and perisinusoidal area.

Results: Immunoexpression of α-SMA was observed in all area with strongest staining on HSCs of perisinusoidal area.

Conclusion: α-SMA can represent a useful marker for early hepatic stellate cells activation in our population and can help target patients requiring aggressive therapy.

KEY WORDS: α-SMA, CHC, liver fibrosis, Hepatic Stellate Cells.

INTRODUCTION

Chronic hepatitis encompasses a group of diseases, typically lasting over 6 months, characterized by varying extent of hepatocellular necrosis and inflammation.1The global burden of hepatitis is high in countries like Egypt, Italy, Romania and Pakistan.[2] The prevalence of hepatitis C in Karachi is 4.2% according to PMRC data of 2009.

Histologically, liver architecture is represented by classic hepatic lobule which has the central vein in the middle while portal tracts are at the periphery. The area around the central vein is termed as pericentral area, while that around portal vein is periportal area.[3]The area of hepatic parenchyma around the sinusoids is the perisinusoidal area. The sinusoids are separated by one cell thick or two cell thick hepatic cords. Between the vascular face of hepatocytes and the endothelial cells lining the sinusoids is a potential space called space of Disse.[4] In this space of Disse reside the hepatic stellate cells, also known as Ito cells. The normal function of HSCs is to store vitamin A. The causative agent of chronic hepatitis C is Hepatitis C Virus which is a single stranded RNA virus belonging to flavivirus family.[5] Once inside the human body, the primary target of HCV is hepatocytes.[6,7] Hepatocytes, in response to injury, secrete various proinflammatory cytokines. The HSCs are in a quiescent state in normal liver.[8] In response to these proinflammatory cytokines the HSCs become contractile and fibrogenic, these HSCs are termed as activated HSCs. [9] These activated HSCs themselves secrete various ECM components and proteases and they lay down collagen leading to fibrosis.[10]This fibrosis, if goes untreated, will progress to cirrhosis and eventually organ failure.[10] Cirrhosis may eventually lead to Hepatocellular carcinoma.[10,11]In Pakistan, almost half of the patients diagnosed with Hepatocellular carcinoma (HCC) are found to be anti-HCV positive.[12] In health care settings of Pakistan, HCV related end stage liver disease, is causing exponential burden on financial health care resources.[12]

These transformed HSCs themselves manufacture several mediators, ECM constituents and proteases, thus leading to fibrosis and cirrhosis.[13]One of the markers expressed by these activated HSCs is Alpha Smooth Muscle Actin (α-SMA), which is a reliable and widely used marker of activated HSCs.[14] However the association between α-SMA and extent of fibrosis is controversial. Several non-invasive techniques are under study for their implication in identification of hepatic fibrosis.[15] Owing to lack of accuracy and dearth of validation, extensive usage of non-invasive methods is not suggested. So far, an impeccable serum marker has not been established.[15]Liver biopsy has been the foundation for diagnosis of hepatic pathologies and denotes the gold standard for assessment of hepatic fibrosis. Liver biopsy is frequently performed under sonographic guidance as an outpatient technique, which paralleled to blind Percutaneous Liver Biopsy(PLB), is not only cost effective, but also curtails the potential perils related to blind PLB.[16] Moreover, biopsy offers supplementary facts about any unrecognized hepatic disease. [17] Hepatitis C viruses (HCV) is an endemic worldwide infection and its distribution and progression varies geographically.[18, 19] Hepatitis C holds several genotypes, 3 being the most prevalent in Pakistan [19] The aim of this study is to appraise the distribution of α-SMA positive HSCs in chronic hepatitis C.

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To find out the frequency of α-SMA positive HSCs in perisinusoidal, periportal and pericentral regions of liver

METHODS

Sixty separate blocks of formalin fixed, paraffin embedded liver biopsy of PCR proven chronic hepatitis C patients was taken. These blocks were collected over a period of year 2010-2012. This Cross sectional study was approved by the Ethics Review Committee of Ziauddin University, Karachi. The biopsies were taken from the BMSI-JPMC and The Laboratory. The study was conducted at Pathology Laboratory, Ziauddin University, Clifton campus, while the test was done at BMSI - JPMC. Sections of 5 µm were cut from the paraffin blocks. The histopathology and immunohistochemistry were then performed.

For Histopathology, H & E stain and Trichome Masson's stain were used. Liver fibrosis was evaluated using Metavir scale. Every specimen was staged for fibrosis on a five-point scale; F0 = no fibrosis; F1 = portal fibrosis without septa; F2 = portal fibrosis with rare septa; F3 = numerous septae without cirrhosis; and F4 = cirrhosis. The activity, which is the amount of necroinflammation, is graded on a 4-point scale from A0 to A3. A0 = no histological activity, A1 = mild activity, A2 = moderate activity, and A3 = severe activity. Score of less than F3 and A2 was taken as low score; a score of F3 and above & A2 and above was taken as high score.[20,21]

For immunohistochemistry, sections were mounted on glass slides coated with poly- L- lysine. Liver sections were incubated with Primary antibody i.e. ready-to-use mouse monoclonal anti α-SMA (Cell Marquee, USA) for 30 min (according to manufacturer's instructions). Positive and negative control slides were included within each session. After incubation with secondary antibody (HRP) and tertiary antibody added (HRP plus) for 20 minutes each, the reaction was visualized using diaminobenzidine. This was followed by counterstaining with Hematoxylin. The immunoexpression of α-SMA on HSCs was scored for periportal, pericentral and perisinusoidal areas.

The total number of HSCs immunostained by α-SMA was determined semi quantitative scoring system. Activated parenchymal HSCs were scored individually in the periportal, perisinusoidal

and pericentral area, where 0: no staining or less than 3 % of the region; I: positive for 3- 33% of the region; II: positive for 34-66% of the region; and III: positive for more than 66 % of the region.[21]

STATISTICAL ANALYSIS

Statistical software SPSS version 20.0 was used for data feeding and analysis. For quantitative variables mean with standard deviation was calculated. For qualitative/ categorical variables percentages and frequencies were calculated. Chi square was applied as test of significance. Only p-value <0.05 was considered to be significant.

RESULTS

Sixty Chronic Hepatitis C cases with the age range of 19 yrs. to 53 yrs were taken. There were 52% males and 48% females (table 1). Their mean age was 37.4+8.4. Most of the CHC patients were observed to be within the age group of 40-49 years.

Table 1: General characteristics

	Number (n)	Percent (%)
Gender		
MAle	29	48.3
Female	31	51.7
Age in years		
Under 30	11	18.3
30-39	18	30
40-49	27	45
50 & above	4	6.7
Necroinflammatory activity		
Low grade (0-1)	22	36.7
High grade (2-3)	38	63.3

Fibrosis		
Low stage (0-2)	37	61.7
High stage (3-4)	23	38.3

The total number of HSCs immunostained by α-SMA was determined semi quantitatively. HSCs were found to be spindle shaped with out-stretched processes and a central nucleus. The cells that showed a nucleus were taken as activated hepatic stellate cells. The α-SMA positive HSCs were detectable in all areas. Periportal, Perisinusoidal and Pericentral areas.

In perisinusoidal area, the immunoexpression of SMA was highest, with most of the cases giving score II i.e.33-66% positivity.(table 2 & 3 ; figure 1) However, the immunoexpression in lower stage of fibrosis (F≤ 2) was significantly higher than the immunoexpression in high stages (table 3; p=0.001). Likewise, in patients with low necroinflammatory grade (A0-A1) showed higher immunoexpression when compared to patients with higher necroinflammatory grade (table 2; p=0.025)

TAble 2: Immunoexpression of α-SMA on HSCs in CHC Patients with grade of necroinflammatory Activity (n=60)

α-SMA expression			Activity Group				P value
			Low Score		High Score		
			n	%	n	%	
Perisinusodial area	0	(less than 3%)	0	0	1	100	0.025
	I	(3-33%)	2	11	16	89	
	II	(34-66%)	19	47.5	21	52.5	
	III	(above 67%)	1	100	0	0	
Periportal area	0	(less than 3%)	0	0	2	100	0.476
	I	(3-33%)	12	40	18	60	
	II	(34-66%)	10	38.5	16	61.5	
	III	(above 67%)	0	0	2	100	
Pericentral area	0	(less than 3%)	6	35.5	11	64.7	0.735
	I	(3-33%)	12	38.7	19	61.3	
	II	(34-66%)	4	40	6	60	
	III	(above 67%)	0	0	2	100	

P value < o.o5 is taken as significant.

TAble 3: Immunoexpression of α-SMA on HSCs in CHC Patients with grade of stage of Fibrosis (n=60)

α-SMA expression	Fibrosis Group				P value
	Low Score		High Score		
	n	%	n	%	

Perisinusodial area	0	(less than 3%)	1	100	0	0	0.001
	I	(3-33%)	4	22	14	78	
	II	(34-66%)	31	77.5	9	22.5	
	III	(above 67%)	1	100	0	0	
Periportal area	0	(less than 3%)	1	50	1	50	0.053
	I	(3-33%)	23	76.7	7	23.3	
	II	(34-66%)	13	50	13	50	
	III	(above 67%)	0	0	2	100	
Pericentral area	0	(less than 3%)	12	70.6	5	29.4	0.215
	I	(3-33%)	20	64.5	11	35.5	
	II	(34-66%)	5	50	5	50	
	III	(above 67%)	0	0	2	100	

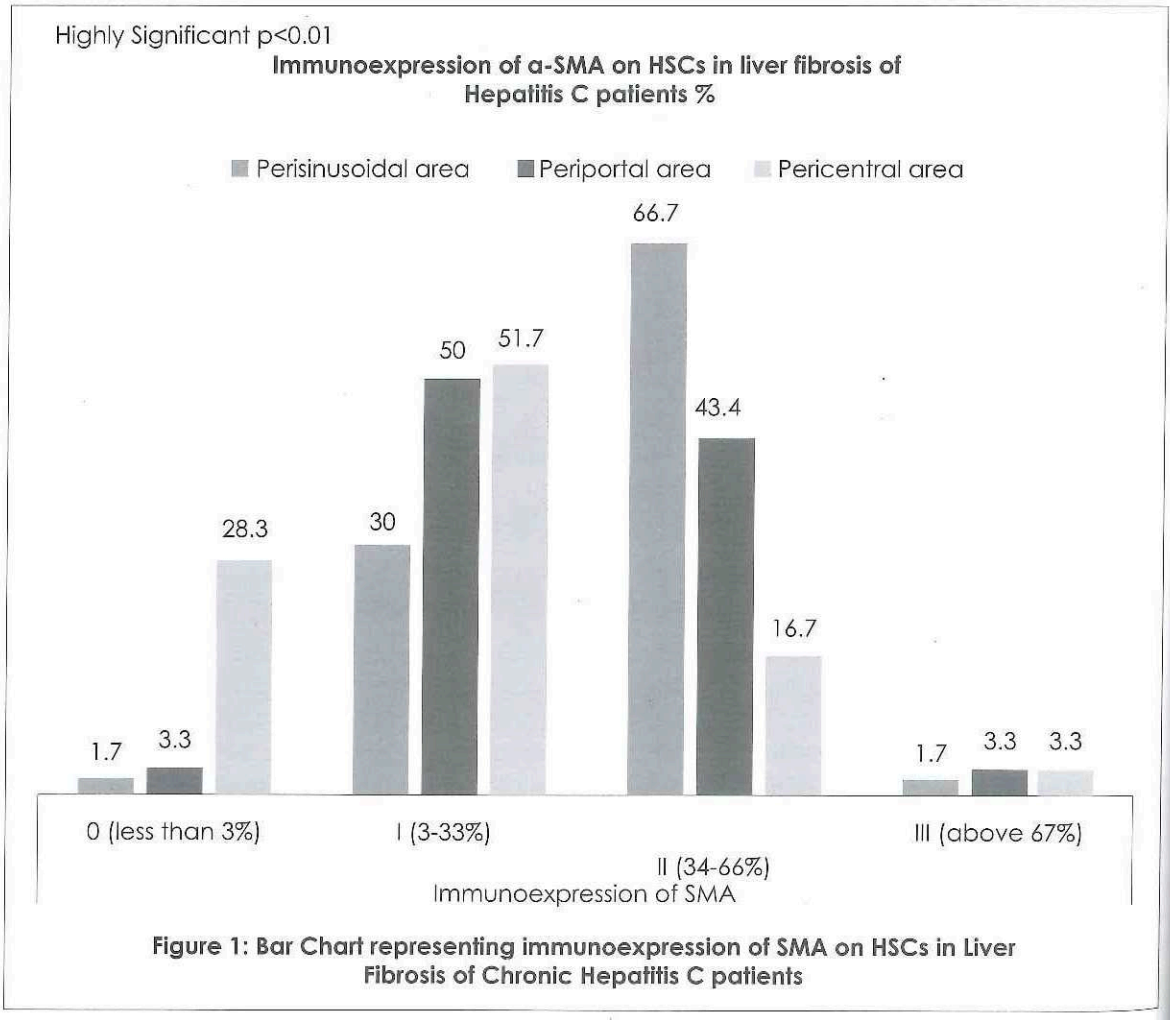

Figure 1: Bar Chart representing immunoexpression of SMA on HSCs in Liver Fibrosis of Chronic Hepatitis C patients

Figure 2: Perisinusoidal α -SMA positive HSCs in a liver section with low Metavir Score (A1F2) & Immunoscore III (> 66 %)
Magnification 200 x approximately.

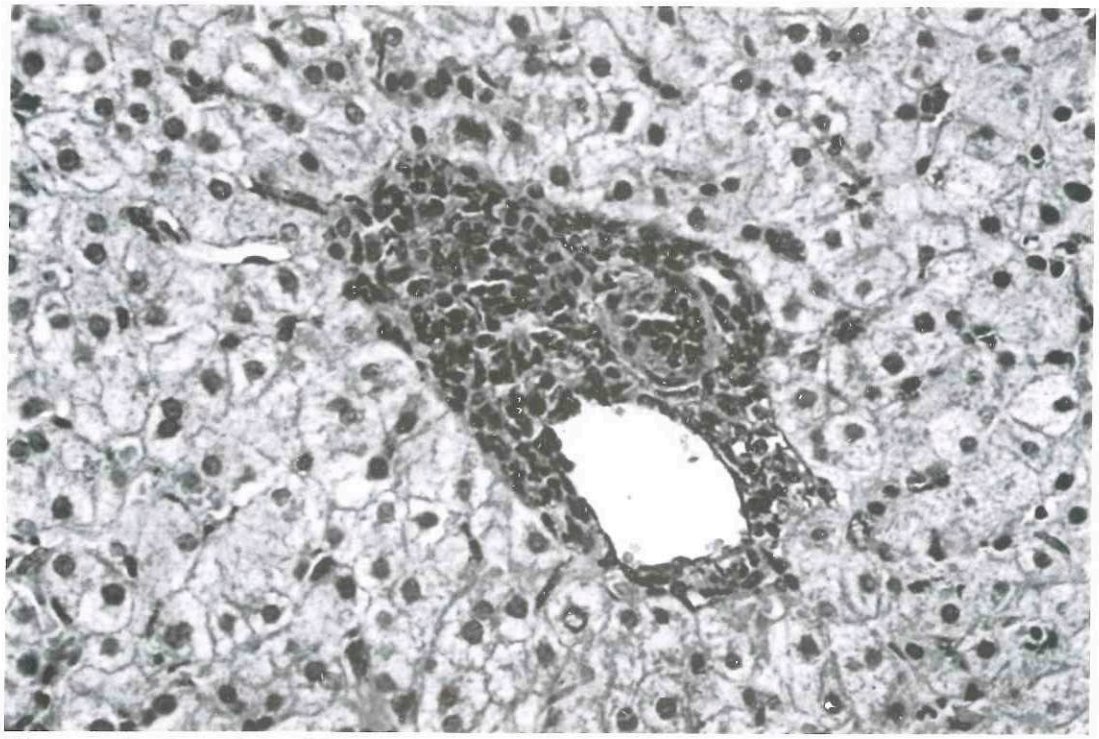

Figure 3: Periportal α -SMA positive HSCs in CHC with low Metavir score (A1F2) and a low immunoexpression {Immunoscore I (3-33%)}
Magnification 400 x approximately.

The immunoexpression in perisinusoidal region was followed by periportal area where most of the cases showed immunoscore of I i.e. 3-33% positivity. (Figure 1 & 2) Pericentral area, however, did not show much of the immunoreactivity. Most of the cases of this area showed immunoscore of I, while 17 % of the cases were observed to have negligible positivity with a score of less than 3 % of the region. (Figure 1)

DISCUSSION

Being a highly prevalent disease in this part of the world, CHC should be diagnosed early to prevent its progress to cirrhosis and eventually organ failure. Liver Fibrosis may not be clinically apparent until an advanced or cirrhotic stage.[22] Liver biopsy has been the cornerstone for identification of liver pathologies and symbolizes the gold standard for assessment of hepatic fibrosis.[23] The Hepatic Stellate Cells are the main contributors of hepatic fibrosis and augmentation of hepatic stellate cells in CHC has made the expression of their markers as reliable immunohistochemical markers of CHC.[21, 24] HSCs activity has been quantified with reference to the magnitude of fibrosis &necroinflammatory activity.[25]The activated stellate cells express mesenchymal markers including α-SMA. [26] The α-SMA expression is strikingly augmented in CHC due to stellate cells activation.[26] However, the association between the α-SMA-positive HSCs and the magnitude of fibrosis is debatable. Our study showed a more prominent immunoexpression of α-SMA positive cells in perisinusoidal areas.[21]This is probably because of the location of HSCs in apposition with sinusoids, leading to prompt response of HSCs to early endothelial modifications after injury. The α-SMA positive HSCs were observed to be significantly detectable in all stages and grades of CHC.[27,28] It was seen that immunoexpression was more significant in lower stage of fibrosis (F≤ 2) than in high stage.[21]The reason for this may be that the activation of HSCs was already triggered by the virus infection, even before appearance of marked fibrosis. Likewise, the expression of α-SMA is higher in lower necroinflammatory grades as compared to the higher grades of necroinflammation.[21,29] These observations are consistent with prior studies.[29]

The activation of perisinusoidal HSCs may be an indicator of probable fibrosis. We can use this marker to judge the activity of HSCs which indicate the chances of fibrosis in an individual.

In conclusion; our study shows that α-SMA can be considered as a useful marker for diagnosis of early hepatic fibrosis in CHC patients and after liver transplant. The patients, in whom HSCs are active, may be considered for target therapy, even without evidence of fibrosis on routine histology.

REFERENCES

1. Fuchs, B. C., Hoshida, Y., Fujii, T., Wei, L., Yamada, S., Lauwers, G. Y., et al. Epidermal growth factor receptor inhibition attenuates liver fibrosis and development of hepatocellular carcinoma. Hepatology, 2014; 59: 1577–1590.
2. Lavanchy D. The global burden of hepatitis C. Liver Int. 2009; 29(1):74–81.
3. Herrine S.K., Navarro V.J., & Rubin R. The liver and Biliary System. In Rubin E, Gorstein F, Rubin R, Schwarting R, Strayer D (eds). Rubin's Pathology: Clinicopathologic Foundations of Medicine. 6th edition. USA: Lippincott Williams and Wilkins; 2012: p 678.
4. Tanaka, M., Itoh, T., Tanimizu, N., & Miyajima, A. Liver stem/progenitor cells: their characteristics and regulatory mechanisms. Journal of Biochemistry, 2011; 149(3): 231-239.
5. Alter,M.J. Epidemiology of hepatitis C virus infection. World Journal of Gastroenterology, 2007; 13(17): 2436.
6. Lavanchy, D., Evolving epidemiology of hepatitis C virus. Clinical Microbiology and Infection, 2011; 17: 107–115.
7. Ryder, S. D., & Beckingham, I. J. ABC of diseases of liver, pancreas, and biliary system: Acute hepatitis. British Medical Journal, 2001; 322(7279): 151.
8. Sibulesky, L..Normal liver anatomy. Clinical Liver Disease, 2013; 2(1): 1-3.
9. D'Ambrosio, R., Aghemo, A., Rumi, M. G., Ronchi, G., Donato, M. F., Paradis, V., et al. A morphometric and immunohistochemical study to assess the benefit of a sustained virological response in hepatitis C virus patients with cirrhosis. Hepatology, 2012; 56: 532–543.
10. Puche JE, Saiman Y and Friedman SL. Hepatic stellate cells and liver fibrosis. Comprehensive Physiology.2013:1473–1492.
11. Mohd. Hanafiah K, Groeger J, Flaxman AD, Wiersma ST Global epidemiology of hepatitis C virus infection: New estimates of age-specific antibody to HCV seroprevalence. Hepatology. 2013;57(4):1333-42
12. Umar M, Bilal M. Hepatitis C, A Mega Menace: A Pakistani Perspective. J Pak Med Stud. 2012;2(2):68-72
13. Pellicoro, A., Ramachandran, P., Iredale, J. P., & Fallowfield, J. A.. Liver fibrosis and repair: immune regulation of wound healing in a solid organ. Nature Reviews Immunology, 2014;14(3): 181-194
14. Fausther M, Lavoie E.G, Dranoff J.A. Contribution of myofibroblasts of different origins to liver fibrosis. Current Pathobiology Reports. 2013; 1(3):225–230.
15. Sebastiani, G., and A. Alberti. How far is noninvasive assessment of liver fibrosis from replacing liver biopsy in hepatitis C. Journal of Viral Hepatology, 2012; 19:18-32.
16. Bravo AA, Sheth SG, Chopra S. Liver biopsy. N Engl Med. 2001;344:495–500.
17. Vijayaraghavan GR, David S, Bermudez-Allende M, Sarwat H. Imaging-guided Parenchymal Liver Biopsy: How We Do It. J Clin Imaging Sci. 2010;1:30.
18. Kanwal, S., & Mahmood, T. Hepatitis C virus resistance to interferon therapy: an alarming situation. Centr European Journal of Biology, 2014;9(12): 1155-1167
19. Sievert, W., Altraif, I., Razavi, H. A., Abdo, A., Ahmed, A., AlOmair, A.et al, A systematic review of hepatitis C virus epidemiology in Asia, Australia and Egypt. Liver International, 2011; 31: 61–80.
20. Alboraie, M., Khairy, M., Elsharkawy, A., Elsharkawy, M Asem, N., El-Seoud, A. R. A. et al. Egy-score as a noninvasive score for the assessment of hepatic fibrosis in chronic hepatitis C: A preliminary approach Saudi journal of Gastroenterology: official journal of the Saudi Gastroenterology Association, 2014;20(3): 170.
21. Sohier Zakaria MY, Moussa M, Akl M, El-Ahwany E, El-Raziky M, Mostafa O, et al. Value of α-smooth muscle actin and glial fibrillary acidic protein in predicting early hepatic fibrosis in chronic hepatitis C virus infection AM 2010;6(3):356-365
22. Baranova A, Lal P, Birerdinc A, Younossi ZM. Non-invasive markers for hepatic fibrosis. BMC Gastroenterology 2011; 11(1):91–105.
23. Patel P, Khan N, Rani M, Gupta D, Jameel S. The Expression of HIV-1 Vpu in Monocytes Causes Increased Secretion of TGF-β that Activates Profibrogenic Genes in Hepatic Stellate Cells. PloS one.2014;9(2)
24. Olsen AL, Bloomer SA, Chan EP, Gaça MD, Georges P, Sackey B, et al. Hepatic stellate cells require a stiff envir

ment for myofibroblastic differentiation. American Journal of Physiology- Gastrointestinal & Liver Physiology. 2011;301(1):110-118
25. Sandulescu, Larisa, I. Rogoveanu, T. Ciurea, Maria Victoria Comanescu, C.T. Streba et al. Immunohistochemical study of stellate cells in patients with chronic viral hepatitis C genotype 1. Romanian Journal of Morphology & Embryology, 2011; 52:137-143.
26. Rockey, D. C., Weymouth, N., & Shi, Z. Smooth Muscle α Actin (Acta2) and Myofibroblast Function during Hepatic Wound Healing. PloS one, 2013;8(10): e77166.
27. Carloni V, Luong TV, Rombouts K. Hepatic stellate cells and ECM in HCC: more complicated than ever. Liver International. 2014
28. Ionescu, A. G., Streba, L. A. M., Vere, C. C., Ciurea, M. E., Streba, C. T., Ionescu, M. I. et al. Histopathological and immunohistochemical study of hepatic stellate cells in patients with viral C chronic liver disease. Rom J Morphol Embryol, 2013; 54(4): 983-991.
29. Carotti S, Morini S, Corradini SG, Burza MA, Molinaro A, Carpino G, et al. Glial fibrillary acidic protein as an early marker of hepatic stellate cell activation in chronic and posttransplant recurrent hepatitis C. Liver Transplantation. 2008;14(6):806-14.